DEPARTMENT OF THE AIR FORCE
AIR FORCE RESEARCH LABORATORY
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28 June, 2000

MEMORANDUM FOR US EPA
NCEA (MD-52)
RTP, NC 27711
ATTN: ANNIE M. JARABEK

FROM: Rebecca Clewell
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SUBJECT: Consultative Letter, AFRL-HE-WP-CL-2000-0037, Preliminary Physiological Model for Perchlorate in the Lactating Rat

1. The Operational Toxicology Branch performed oral dosing studies with pregnant and lactating Sprague-Dawley rats using drinking water treated with perchlorate. The intent of the studies was to evaluate the effect of maternal exposure to perchlorate on the hormone levels in both the dams and the pups, and also to determine the extent of pup exposure via the maternal milk. TSH (thyroid stimulating hormone), free and bound T_4 (thyroxine) and T_3 (triiodothyronine) were measured in the blood of the dams and the pups. Perchlorate was also measured in the serum, thyroid, skin and gut contents of the dams and pups and in the dams' milk. These studies are the basis for the development of a physiologically based pharmacokinetic (PBPK) model for perchlorate in the lactating rat.

2. The PBPK model currently being developed attempts to describe perchlorate dosimetry in the thyroid, serum and milk of the lactating dams from the 1.0 and 10.0 mg/kg/day dose groups. At this time the lower dose groups (0.01 and 0.1 mg/kg/day) of the perchlorate distribution studies (Yu *et al.*, 2000a) are under review. Future model development will attempt to include data from the lower doses and predictions of pup perchlorate levels. The data from several planned and on-going animal investigations will allow future expansion of the PBPK modeling effort (see Attachment).

3. For further information, please contact Rebecca Clewell by phone: (937) 255-5150 x 3141, fax: (937)255-1474 or e-mail: rebecca.clewell@wpafb.af.mil.

REBECCA A. CLEWELL
Operational Toxicology Branch

Attachment: Preliminary Physiological Model for Perchlorate in the Lactating Rat

1st Ind, AFRL/HEST 28 June 2000

MEMORANDUM FOR US EPA
ATTN: MS. ANNIE JARABEK

This letter report has been coordinated at the branch level and is approved for release.

DAVID R. MATTIE, Ph.D.
Acting Chief
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Preliminary Physiological Model for Perchlorate in the Lactating Rat

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INTRODUCTION

Ammonium perchlorate is a powerful oxidizer and the primary component of solid rocket fuel mixtures. It is also present in fireworks, ammunition and commercial fertilizers. Most perchlorate salts are highly soluble in water due to the large surface area and the small, dissociated charge of the anion. Perchlorate, the anion formed by the dissociation of the ammonium salt, has been found in the drinking water supplies of more than 11 states (Urbansky, 1998; Urbansky and Shock, 1999). Contamination of surface and ground water by perchlorate has resulted in concern over the health effects of long term ingestion of perchlorate (Mattie and Jarabek, 1999).

Use of perchlorate dates to the early 20th century, when its potassium salt was prescribed for the treatment of Grave's disease, an advanced form of hyperthyroidism. This treatment was eventually terminated due to complications and reported side effects (Wolff, 1998). However, perchlorate is often used in the investigation of the endocrine system's regulation of iodide. The current understanding of perchlorate's mode of action is that the similar size of the perchlorate ion to that of inorganic iodide allows perchlorate to competitively bind to the Na+/I- symporter in the thyroid, thereby reducing the amount of iodide available in the thyroid for hormonogenesis. The diminished hormone levels signal the pituitary to increase levels of thyroid stimulating hormone (TSH), which in turn stimulates the thyroid to increase iodide symporter activity (Bullock and Rosenthall, 1992; Wolff, 1998). In addition to the thyroid, several tissues show active uptake of iodide and may also be affected by the presence of perchlorate. Other sites of active iodine sequestration include the mammary gland, salivary gland, placenta, skin, ovary and gastrointestinal tract (Brown-Grant, 1961).

During the first two years of human life, a critical window of development exists. During this time the thyroid is responsible for producing hormones which govern physical and mental development (Bakke *et al.*, 1976; Porterfield, 1994). For example, the human brain is only about one third of its final size at birth and it grows rapidly throughout the first two years. During this time brain development is dependent upon thyroid hormones, which in turn are dependent upon the availability of iodide. Infants obtain the necessary iodide through maternal milk. A short-term iodide deficiency during this critical period has been shown to result in lifelong consequences. In humans, gestational and neonatal iodide deficiency and hypothyroidism have been associated with increased numbers of stillbirths, congenital abnormalities, fetal iodide deficiency, lowered IQ, mental retardation and impaired hearing resulting from abnormalities in the inner ear (Delange, 2000; Hetzel, 1989 and Dobbing, 1974 as cited in Gokmen and Dagu, 1995; Porterfield, 1994; Haddow *et al.*, 1999; Klein *et al.*, 1972). In rats, studies have shown developmental hypothyroidism to result in brain cell disorganization and delayed onset of puberty and estrus (Bakke *et al.*, 1976; Clos *et al.*, 1974).

By the twelfth week of gestation in a human (Porterfield, 1994; Roti *et al.*, 1983), or by gestational days 18 to 20 for the rat (Geloso, 1961 as cited in Eguchi *et al.*, 1980), the fetus has a functional thyroid-pituitary axis, is sequestering iodide and is beginning to synthesize and secrete its own hormones (Geloso, 1961 and Nataf and Sfez, 1961 as cited in Eguchi *et al.*, 1980).

Eguchi *et al.* (1980) further contend that a reciprocal relationship (or the thyroid-pituitary feedback) is in place by days 19 through 20 of gestation in the rat. The iodine needed for fetal hormone production is obtained from the mother during gestation. Iodine is thought to pass freely through the human placenta and has been shown to be actively concentrated by the placenta in the rat (Roti *et al.*, 1983; Brown-Grant, 1961).

After birth, the neonate is solely responsible for the production of its thyroid hormones. While small amounts of triiodothyronine (T_3) have been found in milk, very little or no thyroxine (T_4) is present in rat or human milk (Potter *et al.*, 1959; Vigouroux and Rostaqui, 1980; Vigouroux *et al.*, 1980; Sato and Suzuki, 1979; Brown-Grant and Galton, 1958). However, while the infant is nursing, it is dependent upon the maternal milk as the sole source of iodide for hormone synthesis. Iodide, which is actively sequestered in the mammary gland, is then transferred to the pup via the milk primarily in its inorganic form or bound to proteins as hormone precursors, mainly monoiodotyrosine (MIT) and small levels of diiododtyrosine (DIT) (Iino and Greer; 1961; Vigouroux *et al.*, 1980; Brown-Grant and Galton, 1958; Brown-Grant, 1957). Inorganic iodide is thought to be actively sequestered in the mammary gland in a process similar to that of the thyroid and is then organified within the mammary tissue (Brown-Grant and Galton, 1958; Brown-Grant, 1961; Spitzweg *et al.*, 2000).

Maternal exposure to perchlorate in drinking water during pregnancy and lactation raises several concerns which result from the ability of perchlorate to inhibit iodide uptake. It is vital to determine the possible consequences of perchlorate induced hypothyroidism and diminished maternal iodide levels to both the mother and offspring during this critical window of development. It is also necessary to determine the extent to which perchlorate is transferred via the placenta and/or milk during gestation and nursing. Since iodide is actively sequestered in the mammary tissue for transfer in milk, it is possible that perchlorate will actively compete with the inorganic iodide uptake in the mammary gland, yielding less available iodide for lactational transfer. Additionally, it is possible the sequestered perchlorate could be transferred in the milk, resulting in diminished hormone production in the neonate.

The research described in this paper utilizes a physiologically based pharmacokinetic (PBPK) model to describe the movement of perchlorate in the lactating rat after maternal ingestion of drinking water treated with ammonium perchlorate. Mathematical equations are used to describe the uptake and excretion kinetics of perchlorate within the dam and the transfer of perchlorate through maternal milk. This model is an extension of the work performed by Fisher *et al.* (2000) on perchlorate kinetics in the adult male rat. The approach to the modeling of lactational transfer is based on the work done by Fisher *et al.* (1990) with trichloroethylene. The purpose of this paper is to report the progress to date on a PBPK lactation model in the rat. The intent of the model will be ultimately to predict the kinetics of perchlorate in the dam and pup and the pup dose through maternal drinking water exposure during the critical neonatal period.

The model is presently limited to the prediction of perchlorate concentrations in the serum, thyroid and milk in the lactating dam. Predictions are based on the results of a drinking water study performed by AFRL/HEST, in which lactating dams were provided drinking water treated with perchlorate during gestation and up to ten days postpartum (Yu *et al.*, 2000a). Currently, neither inhibition nor perchlorate kinetic data specific to the lactating dam or the nursing pup are

available. Kinetic parameters in this model are based upon radiolabeled perchlorate ($^{36}ClO_4$) studies in the adult male rat (Yu *et al.*, 2000b) and the concurrent modeling effort of Fisher *et al.* (2000). This paper describes the present status of model development for the lactational transfer of perchlorate exclusively in the lactating dam.

METHOD

Supporting Experiments

Experiments used in model development were performed at AFRL/HEST. Pregnant dams began exposure to drinking water treated with perchlorate on gestational day (GD) 2. Dams were continued on daily drinking water exposure throughout gestation and lactation. Both dams and pups were sacrificed on either PND 5 or 10 and serum and thyroids were harvested and analyzed for perchlorate, free and total thyroxine (fT_4 and tT_4), triiodothyronine (T_3) and TSH. Milk was also collected from the dams and analyzed for perchlorate concentrations. These studies are described in detail in another report (Yu *et al.*, 2000a).

Analytical Method

Serum and milk samples were prepared in the following manner. A portion of the sample (100 µL) was precipitated with 400 µL of ice cold 100% ethanol. Samples were then centrifuged in a refrigerated Eppendorf microcentrifuge at 17,000 rpm for 30 minutes at 4°C. The supernatant was subsequently drawn off and evaporated to dryness under nitrogen at 37°C. Dried samples were reconstituted in 2 mL distilled deionized water (18.3 MΩ/cm) and filtered with 0.45 µm Acrodisc (Pall Gelmann Laboratory, Ann Arbor, MI) filters. Further dilutions were made according to the dose group from which the samples were obtained. Aliquots (50, 300, 600 and 1000 µL) of the reconstituted samples obtained from the 10.0, 1.0, 0.1 and 0.01 mg/kg-day dose groups, respectively, were diluted to a final volume of 2 mL.

Thyroid samples were prepared by adding 250 µL of water to the tissue and homogenizing with micro tissue grinders (Kontes Company, Vineland, NJ). Homogenates were centrifuged twice at 17,000 rpm x G for 30 minutes at 4°C. The supernatant was then diluted 12.5, 40, 80 and 250 times with distilled, deionized water for 0.01, 0.1, 1.0 and 10.0 mg/kg-day dose groups, respectively. Samples were subsequently filtered with 0.45 µm Acrodisc filters (Pall Gelmann Laboratory, Ann Arbor, MI) and analyzed with ion chromatography.

Ion chromatography was performed on a Dionex DX-300 (Dionex Corp., Sunnyvale CA) system equipped with an AGP advanced gradient pump, an AS-3500 autosampler and a CDM-3 conductivity detector. Separation was obtained with an AS-11 (4 mm) analytical column and an AG-11 (4 mm) guard column. The mobile phase consisted of 100 mM NaOH, flowing at 1.0 mL/min. Background conductivity was minimized with an ASRS-Ultra (4 mm) self-regenerating suppressor set to 500 mA, with external water flowing at 10 mL/min. Sample loop

volume was 1 mL. Chromatography was analyzed with Peaknet 5.1 software (Dionex Corp., Sunnyvale CA).

Model Development

This model remains consistent with concurrent model development efforts for the adult human and the adult male rat (Merrill *et al.*, 2000; Fisher *et al.*, 2000). While it is understood that many physical and hormonal changes are present during the period of lactation and neonatal development, the PBPK model was developed with the assumption that the lactating dam could be described in the same basic manner as the male rat. The lactation model consists of two lumped compartments (slowly and richly perfused tissues), a lumped blood compartment and separate two-compartment models for the tissues that actively sequester perchlorate (thyroid, skin and gut). However, in the lactational transfer model, it was necessary to include an additional compartment for the mammary blood and milk production. Additional accounting was necessary for the growth of mammary tissue during lactation and the changing loss of perchlorate to the suckling pups. The schematic for the proposed model is shown in Figure 1.

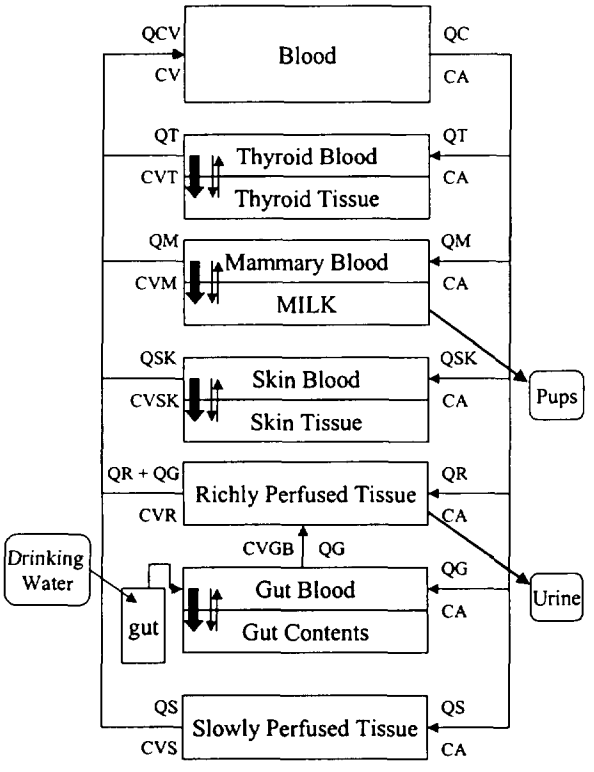

Figure 1. Schematic for Perchlorate Model in the Lactating Dam. Bold arrows indicate active uptake into a compartment. Small arrows indicate passive diffusion between compartments.

The mammary compartment was described using two sub-compartments, mammary blood (serum) and milk. In order to maintain simplicity, the perchlorate was assumed to be taken up into the milk directly from the mammary blood. A Michaelis-Menton term for saturable uptake was utilized to simulate the active uptake of perchlorate into the milk, along with first order rates of passive diffusion between the milk and mammary blood. Since the volume of mammary tissue and the fractional blood flow to the mammary tissue are increasing throughout the period of lactation, it was necessary to account for the growth of these tissues over time. A table function in ACSL (Advanced Continuous Simulation Language, Pharsight Corp., Mountain View, CA) was employed in order to account for these tissue changes. This function employs simple linear extrapolation between literature data points in order to estimate the value of the parameter at any point in time. With each iteration of the model, all tissue and blood flow values are adjusted with respect to the changing mammary tissue. Increasing suckling rate was also simulated using a table function in ACSL.

Whenever possible, literature and experimental values were used for physiological parameters. Those not available in the literature or through experimentation were calculated from values for adult male rats using allometric scaling. Allometric scaling for maximum velocity (Vmax), first order rate constants (K), tissue volumes (V) and blood flows (Q) are shown in Equations 1 through 4, where X is the tissue of interest and BW is the average body weight of the rat. A value of C following the parameter name indicates the value of a parameter before allometric scaling.

$$VmaxX = VmaxXc \times BW^{3/4} \qquad \text{(Equation 1)}$$

$$KX = KXc / BW^{1/4} \qquad \text{(Equation 2)}$$

$$VX = VXc \times BW \qquad \text{(Equation 3)}$$

$$QX = QXc \times BW^{3/4} \qquad \text{(Equation 4)}$$

The body weight of the dam was calculated separately for each dose from the drinking water study (Yu *et al.*, 2000a) using the average weight of the dams for each dose group for PND 1 through 10. Km (Michaelis-Menton affinity constant) values for perchlorate were set equal to those of iodide, which were obtained from the literature. Values for Km were found to be similar across species and laboratories (Gluzman and Niepomniszcze, 1983; Wolff and Maurey, 1963) and Km values for non-thyroidal tissues were similar to those in the thyroid (Wolff and Maurey, 1961). However, the maximum velocity (Vmax) has been found to vary between tissues (Wolff and Maurey, 1961). As a result, Km values were held constant and the values for Vmax in the various compartments were obtained by fitting the model simulation to experimental data. Tables 1 and 2 list the physiological and chemical specific parameters employed within the model and their sources.

TABLE 1. PHYSIOLOGICAL PARAMETERS

Parameter	Value	References
Volumes		
Body Weight of the Dam *BW* (kg)	0.2839-0.2923	Yu *et al.*, 2000a
Plasma V*plasc* (%BW)	4.66	Brown *et. al.* 1997, Altman and Dittmer, 1971
Mammary *VMc* (%BW)	4.4-9.6	Knight *et al.*, 1984
Milk *Vmk* (L)	0.002	Knight *et al.*, 1984
Thyroid *VTc* (%BW)	0.0065	Unpublished data, Yu *et al.*, 2000b
Thyroid Capillary Blood *VTBc* (%VT)	18.1	18.1% thyroid weight in rat (Brown *et al.*, 1997)
Skin *VSkc* (%BW)	19	Brown *et al.*, 1997
Skin Capillary Blood *VSkbc* (%VSk)	2	Brown *et al.*, 1997
Gut (without contents) *VGc* (%BW)	3.6	Brown *et al.*, 1997
Gut Capillary Blood *VGBc* (%VG)	2.9	Altman and Dittmer, 1971
Slowly Perfused *VS* (L)	0.1390 – 0.1625	Lumped compartment (calculated from Brown *et al.*, 1997)
Richly Perfused *VR* (L)	0.0145 – 0.0164	Lumped compartment (calculated from Brown *et al.*, 1997)
Blood Flows		
Cardiac Output *QCc* (L/hr/kg)	14	Brown *et al..*, 1997
Mammary *QMc* (%QC)	9.0-15.0	Hanwell and Linzell, 1973
Thyroid *QTc* (%QC)	1.6	Brown *et al..*, 1997 (human)
Gut *QGc* (%QC)	13.6	Brown *et al.*, 1997
Skin *QSKc* (%QC)	5.8	Brown *et al.*, 1997
Slowly Perfused *QS* (L/hr)	1.162 – 1.307	Lumped compartment (calculated from Brown *et al.*, 1997)
Richly Perfused *QR* (L/hr)	3.682 – 4.126	Lumped compartment (calculated from Brown *et al.*, 1997)

TABLE 2: CHEMICAL SPECIFIC PARAMETERS

Partition Coefficient (unitless)	**Value**	**Source**
Slowly perfused / plasma *PS*	0.31	Yu *et al.*, 2000b, Fisher *et al.*, 2000
Rapidly perfused / plasma *PR*	0.56	Yu *et al.*, 2000b, Fisher *et al.*, 2000
Max Capacity, *Vmaxc* (ng/hr/kg)		
Thyroid *VmaxTc*	5.0E3	Fitted
Mammary *VmaxMc*	1.75E5	Fitted
Skin *VmaxSkc*	1.0E8	Fitted, Yu *et al.*, 2000b, Fisher *et al.*, 2000
Gut *VmaxGc*	3.8E4	Fitted, Yu *et al.*, 2000b, Fisher *et al.*, 2000
Affinity Constant, *Km* (ng/L)		
Thyroid *KmT*	3.96E6	Gluzman and Niepomniszcze, 1983
Skin *KmS*	3.96E6	Gluzman and Niepomniszcze, 1983
Gut *KmG*	3.96E6	Gluzman and Niepomniszcze, 1983
Milk *KmM*	3.96E6	Fitted
First Order Rate Constant, *K* (/hr)		
Urinary excretion *KUc*	0.5	Fitted
Thyroid blood to thyroid *KTc*	2	Fitted
Thyroid to thyroid blood *KTc*	2	Fitted
Gut content to gut blood *KGc*	100	Fitted
Mammary blood to milk *KMkinc*	4.0	Fitted
Milk to mammary blood *Kmkoutc*	4.0	Fitted
Suckling rate *KSucc*	.0009-.0018	Knight *et al.*, 1984
Skin blood to skin *KSkc*	100	Fitted, Yu *et al.*, 2000b, Fisher *et al.*, 2000
Skin to skin blood *KSkc*	100	Fitted, Yu *et al.*, 2000b, Fisher *et al.*, 2000

The movement of perchlorate into the slowly and richly perfused tissue was accounted for with partition coefficients measured by AFRL/HEST (Fisher *et al.*, 2000b). The thyroid, skin and gut tissues as well as milk were assumed to have both passive diffusion and active uptake of perchlorate. Passive diffusion was handled in the model through the use of first order rates in and out of the tissue of interest. Since time-course data are not available for perchlorate in the lactating rat, passive diffusion rate constants from the adult male rat inhibition model were used (Fisher *et al.*, 2000). These first order rate constants were based on a radiolabeled perchlorate (^{36}Cl) study (Yu *et al.*, 2000b) and were found by obtaining the best fit to perchlorate as it cleared from the tissue of interest.

A Michaelis-Menton term for saturable transfer was used in the skin, mammary, thyroid and gut contents compartments to account for the active uptake of perchlorate. Equations 5 through 7 show an example from the thyroid compartment, where RATB (Equation 5) is the rate of change in amount of perchlorate in the thyroid blood, QT is the flow of blood to the thyroid capillary bed and CA and CVTB are the concentrations of perchlorate in the arterial and thyroid blood, respectively. RupT (Equation 6) is the rate of active uptake into the thyroid tissue and RinT and RoutT are the first order rates of diffusion in and out of the thyroid tissue from the capillary blood. Equation 7 describes the rate of change in perchlorate concentration in the thyroid tissue (RAT). The amount of perchlorate at any one time would then be calculated by integrating RAT.

$$RATB = QT*(CA-CVTB) - RinT + RoutT - RupT \qquad \text{(Equation 5)}$$

$$RupT = (VmaxT*CVTB) / (KmT+CVTB) \qquad \text{(Equation 6)}$$

$$RAT = Rupt + RinT - RoutT \qquad \text{(Equation 7)}$$

In order to simulate the daily dosing regimen of the drinking water experiment, the rats were assumed to drink at constant rate for 12 of the 24 hours per day (1800 to 0600 hours). A pulse function was used to introduce drinking water to the stomach of the rat for the first 12 hours of each 24 hour period and then to stop dosing while the rat was presumably sleeping. The stomach (shown as "gut" in Figure 1) functioned as a theoretical "holding" area within the model from which the gut blood received the perchlorate through first order uptake. Pups, however, were assumed to be nursing at a constant rate, 24 hours a day. This assumption is based on the fact that nursing rats are unable to go for long periods of time without suckling. The loss through suckling was then considered to be a constant first order loss from the mother's milk to the gut of the pup based on the experiments of Knight *et al.* (1984). The milk production rate was assumed to be equal to the amount of milk ingested by the pups.

Urinary excretion has been shown to be the major path of elimination of perchlorate (Wolff, 1998; Fisher *et al.*, 2000). For simulation purposes, the loss through urinary excretion was assumed to take place at a constant rate throughout the day. The first order rate constant for urinary excretion (KUc) was obtained by adjusting the value of KUc in order to gain the best fit to experimental serum levels, since urine data were not available for the lactating rat. Fecal excretion of perchlorate is assumed to be negligible (Fisher *et al.*, 2000).

Estimated Pup Perchlorate Exposure

Although the model has not yet been used to predict pup exposure to perchlorate, predicted pup dose was calculated from experimental milk concentrations obtained from dams in the drinking water study (Yu *et al.*, 2000a). The measured concentration of perchlorate in the milk from the 1.0 and 10.0 mg/kg-day dose groups was used to predict the calculated daily dose of perchlorate to the pup based on maternal ingestion of ammonium perchlorate. In accordance with Knight *et al.* (1984), the milk yield per day (MY) was assumed to be equal to the amount ingested by the pups and was calculated by multiplying the average litter suckling rate (0.0018 L/hr) by 24 hours. In order to maintain consistency with the study performed by Knight *et al.* (1984), an average litter was assumed to have seven pups. The litter weight (BW_L) was then calculated by obtaining the average pup weights on PND 10 from the drinking water study for each of the two dose groups and multiplying by the number of pups (7). The average concentration of perchlorate in the milk (C_{MK}) was then multiplied by the average milk yield per day. The resulting number was then divided by the litter weight (BW_L) in order to obtain the estimated dose to the pup per day, as is shown in the following equation.

$$C_{MK} * MY / BW_L = pup\ dose\ (mg/kg\text{-}day) \qquad \text{(Equation 8)}$$

This estimation for the pup dose is reasonable as long as the loss of perchlorate through the milk does not significantly decrease the serum levels of perchlorate. Although urinary excretion is the primary source for systemic clearance of perchlorate, from the milk and serum perchlorate concentrations found in Yu *et al.* (2000a) it is questionable whether the loss through milk is a significant source of clearance in the dam at the lower doses. Milk and serum values in the 0.01 and 0.1 mg/kg-day dose groups are currently being reviewed to determine the analytical or biological basis for the values obtained. Therefore, this calculation was not applied to either of the lower dose groups.

Theoretical calculations were also performed in order to determine the amount of perchlorate ingested by the dam in a day and the amount ingested by the dam's litter. The amount ingested by the dam was calculated by multiplying the estimated average daily dose (mg/kg-day), determined by water consumption, by the average weight of the dam. The average weight of the dam was found by averaging the daily weights of all dams in the dose group. The amount ingested by the litter was calculated by multiplying the suckling rate (Knight *et al.*, 1984) by 24 hours and the average concentration of perchlorate measured in the milk of the dams from each dose group.

RESULTS

The model simulation was able to successfully predict the serum, thyroid and milk perchlorate levels in the dam for the 1.0 and 10.0 mg/kg-day dose groups. Figures 2 through 7 show the model simulation plotted against the experimental data for PND 5 and 10 for the 1.0 and 10.0 mg/kg-day dose groups in the drinking water study. The model was able to fit serum, milk and thyroid concentrations at both dose groups with one set of parameters.

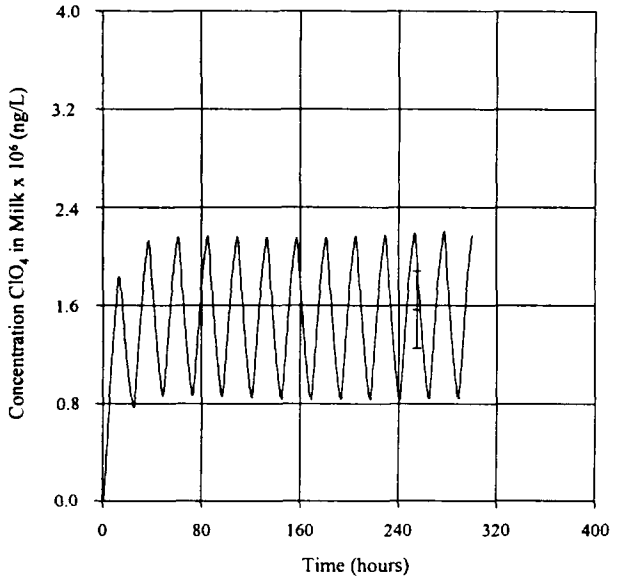

Figure 2. Model predicted (lines) and actual values, including mean and s.d. (bars), of milk perchlorate concentrations from dams treated with 1.0 mg ClO_4/kg-day

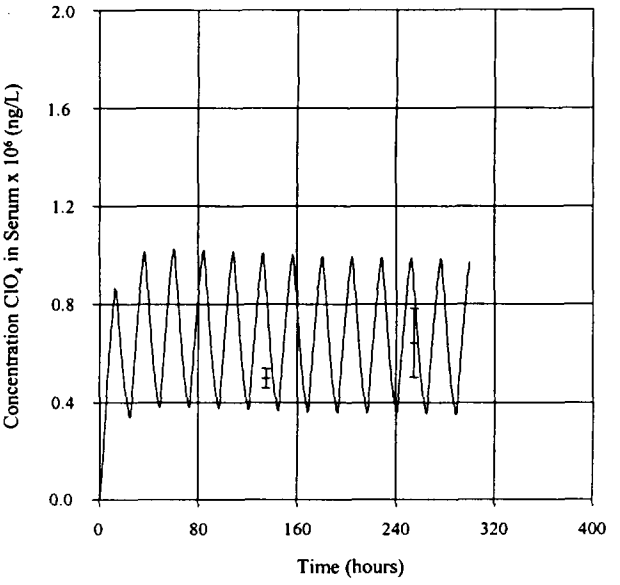

Figure 3. Model predicted (lines) and actual values, including mean and s.d. (bars), of serum perchlorate concentrations from dams treated with 1.0 mg ClO_4/kg-day

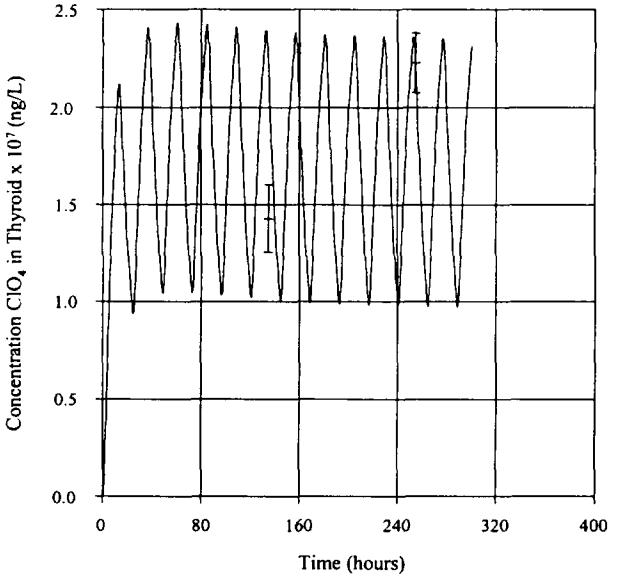

Figure 4. Model predicted (lines) and actual values, including mean and s.d. (bars), of thyroid perchlorate concentrations from dams treated with 1.0 mg ClO_4/kg-day

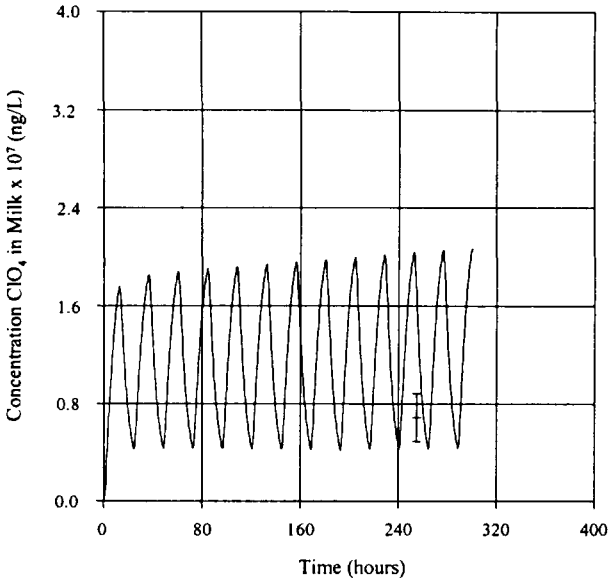

Figure 5. Model predicted (lines) and actual values, including mean and s.d. (bars), of milk perchlorate concentrations from dams treated with 10.0 mg ClO_4/kg-day

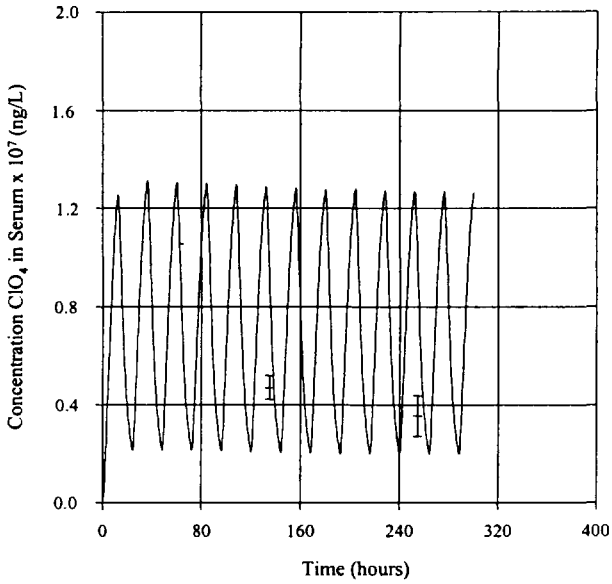

Figure 6. Model predicted (lines) and actual values, including mean and s.d. (bars), of serum perchlorate concentrations from dams treated with 10.0 mg ClO_4/kg-day

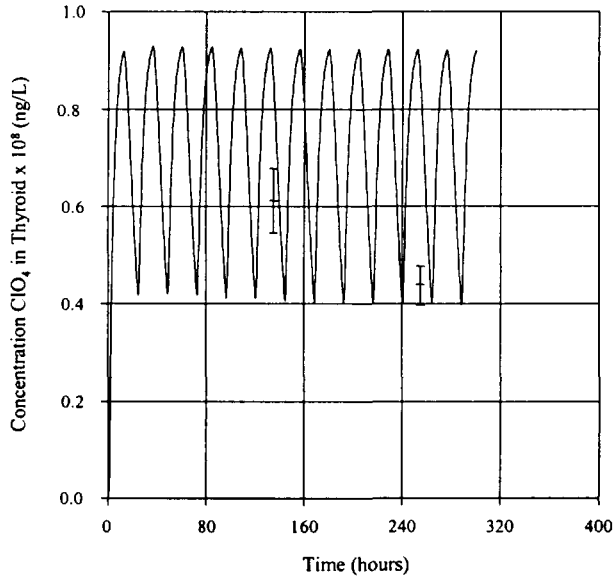

Figure 7. Model predicted (lines) and actual values, including mean and s.d. (bars), of thyroid perchlorate concentrations from dams treated with 10.0 mg ClO_4/kg-day

Active uptake was described in the thyroid by fitting the model prediction to experimental serum and milk perchlorate concentrations (Yu *et al.*, 2000a). KmT was set to 3.9E+6 ng/L from Table 2 and VmaxTc was found to be 25000 ng/kg/hr from fitting of the data. Passive diffusion in and out of the thyroid was described with a first order rate constant (KTc) of 4.0 /hr. Active uptake in the skin was found by adjusting the value to fit serum, thyroid and milk data and was predicted to have a VmaxSkc of 1.0E+8 and a KmSk of 3.9E+6. The first order rate constant for diffusion in and out of the skin (KSkc) was set to 100 /hr. Active uptake into the gut was also determined by fitting the simulation to serum, thyroid and milk data. The fitted value for VmaxGc was 1.0E+8 and KmG was 3.9E+6, while the passive diffusion was described with a KGc of 100 /hr. Milk was found to have a larger maximum velocity than the thyroid. A VmaxMc of 175,000 was found to provide the best fit of the simulation to experimental serum and milk perchlorate levels; KmM was set to 3.9E+6 in order to maintain consistency with the other tissues and the diffusion was described with a KMkinc and KMkoutc of 4.0 /hr. The urinary excretion was assumed to occur quickly based on studies in the male rat (Yu *et al.*, 2000b). The value for the first order urinary excretion constant (KUc) of 0.8 /hr was found by fitting the serum levels in the lactating dam.

Values obtained with the lactation model agree reasonably with those found in the adult male rat model, which does fit time-course data obtained from the administration of radiolabeled iodide and perchlorate. Values for the Vmax in the thyroid (VmaxTc) and the urinary excretion (KUc), however, were somewhat higher in the lactation model than in the male rat model in order to fit the experimental data.

Estimated Pup Perchlorate Exposure

The estimated maternal dose of perchlorate, amount of perchlorate transferred to the litter and an individual pup dose are shown in Table 3. From the calculations, it is apparent that a significant amount of the maternal dose is transferred to the pup via suckling. This transfer is also supported by the fact that perchlorate levels in pup serum were found to approach those of the lactating dam in the drinking water study (Yu *et al.*, 2000a).

TABLE 3. CALCULATED MATERNAL AND PUP DOSE

Maternal Dose (mg/kg-day)	Amount Ingested by Dam (mg)	Amount Ingested by Litter (mg)	Pup Dose (mg/kg-day)
1.0	0.284	0.068	0.490
10.0	2.923	0.297	2.196

DISCUSSION

The described PBPK model is able to predict serum, thyroid and milk perchlorate concentrations in the lactating rat at high doses of perchlorate in drinking water. Experimental data for tissue concentrations fall within model predictions for both the 1.0 and 10.0 mg/kg-day dose groups. Kinetic parameters, such as the maximum velocity and affinity constants for active uptake into the thyroid, skin and gut, were kept consistent with those obtained through concurrent modeling efforts in the male rat (Fisher *et al.*, 2000).

It is possible to use theoretical calculations to predict pup dose via maternal milk in the high dose groups. The predicted values indicate that the pup receives a significant amount of perchlorate through suckling, which supports the relatively high levels of perchlorate found experimentally in the pup serum (Yu *et al.*, 2000a). It is also possible that, like iodine (Samuel and Caputa, 1965 as cited by Beltz and Reineke, 1967), the pups are not able to excrete perchlorate as efficiently as adults through urine, which could help to explain the elevated perchlorate concentration in their serum.

Although the milk to plasma ratio for perchlorate remains constant across the four dose groups, the relatively high levels of perchlorate in the serum and milk of the dam at the lower doses (0.01 and 0.1 mg/kg-day) (Yu *et al.*, 2000a) appear to indicate that the milk presents a significant loss of perchlorate at the lower doses. Despite the fact that the estimated pup dose could not be calculated at this time, both the non-linearity of the experimental data and the estimated pup exposures in the higher dose groups illuminate the need for further advancements in modeling to accurately predict the pup exposure at all dose levels.

Future improvements of this model to predict lower doses, time-course data, inhibition and hormone data are dependent upon both current and future studies. Although no pup simulations are presented in this paper, work is presently underway in which the pup is being added to the model in order to predict serum levels in the growing pup. Future animal studies will also be useful in the development of the pup model.

It is our goal to eventually include both iodide inhibition and hormonal changes in both the lactating mother and the developing pup. A hormone model for the adult male rat is currently under development at AFRL/HEST. This hormone model will then be used as the basis for the inclusion of hormones in the lactational transfer model described here.

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